THE SUPPRESSOR-MUTATOR SYSTEM OF CONTROL OF GENE ACTION IN MAIZE

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Continued attention has been given during the past year to the manner in which controlling elements in maize affect the phenotypic expression of known genes. Controlling elements, because of their capacity for transposition, are considered as chromosome components apart from the genes, which appear to be stationary. Once this distinction had been made it was possible to examine the mode of operation of controlling elements independently of genes and to study relations among them. It was found that some elements interact with others to control gene expression. In this respect, the elements can be segregated into groups whose members interact with one another but not with members of other groups. Each group exhibits a particular type of control of gene expression. Therefore, a group of interacting elements has been referred to as an operational system. Progress has been made in interpreting the mode of operation of several of these systems, but some important questions about controlling elements themselves remain unanswered. We do not know, for example, how they are incorporated in a chromosome or by what process transposition is accomplished. Experiments aimed at answering such important questions are needed. My attention has not been turned in these directions, because I believe that we should first expand our knowledge of different systems of controlling elements in order to learn, if possible, whether the modes of operation of all controlling elements are basically alike. It is conceivable that these elements may be composed of one common type of substance and have one common mode of operation, as is now thought to be true of genes.

Appreciation of the way in which some systems operate may be gained rapidly; with other systems, analysis may be difficult and progress slow. A system may be responsible for the appearance of an array of quite different phenotypes, even within a single plant. Also, in some cases, analyses may be complicated by what appears to be an irregular form of inheritance of components of the system. That apparent complexities in phenotypic expression can be resolved into ordered patterns when the operation of a component of a system is comprehended became apparent during the course of studies conducted this year; the findings will be considered in the discussion that follows.

The Mode of Operation of the Spm Element

The relation between a_1^{m-1}, located in chromosome 3, and a_2^{m-1}, located in chromosome 5, was considered in Year Book 56. It was mentioned there that the action of genic substance at each of these two loci is under the control of elements belonging to the *Spm* (Suppressor-mutator) system, and, further, that the *Spm* element in a_2^{m-1}

cultures does not behave quite like that in a_1^{m-1} cultures. During the past year, differences between these two elements have been examined. As a consequence, some previously puzzling aspects of the system of control of gene expression in a_2^{m-1} cultures have been clarified.

The difficulties encountered in earlier attempts to analyze the system of control of gene expression at a_2^{m-1} were not experienced in the analysis of a_1^{m-1}. Whereas in the a_2^{m-1} cultures detection of the *Spm* element was much delayed, *Spm* was easily identified in the a_1^{m-1} cultures. Its mode of operation in these cultures could be readily discerned, and its inheritance behavior did not seem complicated. Transposition of the element from one location to another in the chromosome complement could also be followed easily. It was learned, however, that this *Spm* element is not altogether stable. Some changes in its action were recognized; and one of these, effecting a reduction in its capacity to inhibit gene expression at a_1^{m-1} and to cause mutation at that locus, was discussed in Year Book 56. This finding suggested the possibility of a direct relation between the degree of suppressive capacity of an *Spm* element and its ability to induce mutation.

Most of the studies of control of gene expression at a_1^{m-1} were conducted not with the modified *Spm* element, just mentioned, but with the originally identified *Spm* element, whose capacity to suppress the expression of gene action at a_1^{m-1} is complete. In the presence of this *Spm* element there is no visible evidence of action of the genic substance of the a_1^{m-1} locus except in those areas of a plant or kernel that arise from cells in which mutation to or toward A_1 has occurred. The cells having such mutations contain anthocyanin pigment; and the mutants so formed are thereafter stable. The phenotype attributable to a particular mutation will be produced by the mutant either in the presence or in the absence of *Spm* in subsequent generations of plants.

It was found that another type of change also can occur at the locus of a_1^{m-1}. Each such change is reflected in an altered response of a_1^{m-1} to given conditions in subsequent cell and plant generations. The alteration must modify some component of the locus that is capable of being reproduced during each chromosome replication; in other words, the modification effects a heritable change in the state of the locus. Control of the time of occurrence of mutation during development of a tissue, of the type of mutation, and of the number of cells in which mutation takes place, was found to reside at the locus of a_1^{m-1}, each state expressing a particular mutational pattern in the presence of *Spm*. The pattern peculiar to each state is not altered by increased doses of *Spm*. The same pattern appears when one or more than one *Spm* element is present. In the absence of *Spm*, on the other hand, some gene action at a_1^{m-1} is expressed as anthocyanin pigmentation in both plant and kernel by all but one of the examined states, and the degree of this action, as indicated by the intensity of pigmentation, is also a reflection of the state of a_1^{m-1}. In the absence of *Spm*, however, no mutations occur and each state retains its integrity through successive plant generations. In short, the expression of types of mutation and of their pattern of distribution in plant or kernel in the presence of *Spm*, and also the type of gene action exhibited in its absence, define a state of a_1^{m-1}. No unresolved ambiguities in this respect were encountered during an extensive study of states of a_1^{m-1}.

In the a_2^{m-1} cultures, in contrast, such clear-cut distinctions between states were not evident in the early studies. It was realized, nevertheless, that not all derivatives of the original a_2^{m-1} were alike. At first, the different isolates could be segregated only into two main classes. Difficulties were encountered in interpreting the mode of control of gene expression of members within each class. A few examples will be given of the irregular pat-

terns of gene expression observed in members of the first class.

In plants carrying isolates of a_2^{m-1} belonging to the first class, it was noted that abruptly initiated changes of some kind, apparently occurring within individual cells, resulted in the appearance of well defined areas in which the phenotypic expression of gene action at a_2^{m-1} differed from that in the surrounding tissue. Such areas also appeared in ears, all the kernels within an area exhibiting the modified type of gene expression. Again, such modified areas were often seen even within a single kernel. Different types of modification were sometimes observed in the same plant. Anthocyanin pigment would be totally absent in some areas whereas most of the rest of the plant was deeply pigmented; or the intensity of pigment within an area might simply be reduced. Areas were also noted in which streaks of deep pigmentation appeared in an otherwise nonpigmented background. Both the number of these deeply pigmented streaks and their size seemed to be controlled in some manner, for each such area exhibited a precise pattern in these regards. The patterns differed among the areas, however, and often over a very wide range. Some areas had only a few small pigmented streaks; others, many such small streaks. In still others, the size of the streaks was much larger, and there might be either a few or many within one area. In the course of study of isolates belonging to the first class, it was found that mutation to or toward A_2 occurred, but only in those cells of an area that otherwise exhibited no anthocyanin pigment. It was also found that the mutants were thereafter stable with respect to the phenotypes they produced. No variegated phenotypes appeared in either a plant or a kernel having one of the mutations, when tested under conditions that would be expected to reveal any instability of expression had it occurred.

Often, in plants showing the above-described irregular patterns of distribution of anthocyanin pigment, similar irregularities were noted among the kernels. Some kernels were uniformly pigmented, and in most of them the intensity of pigmentation was low. Others were variegated, exhibiting deeply pigmented spots in a colorless background. Sometimes all the variegated kernels on an ear exhibited similar patterns with respect to size and number of such spots, but on other ears wide differences were observed among the variegated kernels. Some had only a few small specks of deep pigmentation, others had many such specks; in some, a number of medium-sized pigmented spots appeared, and in still others there were relatively few spots but most of them were large. Very often, on an ear produced from a testcross, the ratio of variegated to pale-colored kernels, and also the proportions of different kinds of variegated kernels, gave no evidence of orderly meiotic segregation of heritable elements that might be responsible for the appearance of the different kinds of kernels. On some other ears, in contrast, the ratio of kernel types was quite consistent with an orderly segregation. Frequently, the different ears produced by one plant were not at all consistent in this regard, even when the same pollen parent had been used in making the cross to each of the ears.

Confused impressions of the operation of the system of control of gene expression at a_2^{m-1} also resulted from attempts to analyze the constitution of plants derived from the pale-colored kernels on ears that segregated both pale-colored and variegated kernels. Some of these plants had variegated phenotypes. Others, however, showed no evidence of variegation, the anthocyanin pigment being uniform in intensity and distribution throughout the plant. Study of the progeny of these plants led to further difficulties of interpretation. When the ears of one such plant were used in crosses with tester plants homozygous for the stable recessive, a_2, all the pigment-containing kernels on each ear of one plant might be uniformly pale colored; whereas, in another plant, one ear

might have all uniformly colored kernels and another ear might have also a few variegated kernels. These variegated kernels exhibited deeply pigmented spots in a colorless background; the pattern of spots might be the same among all, or might differ widely. On still another ear of the same plant, a clear-cut Mendelian ratio of pale to variegated kernels might appear.

That some system of control of gene expression was operating in the case of a_2^{m-1} was clear, but evidence from the early studies did not allow ready recognition of its components. The above-described examples of irregular phenotypic expressions and inheritance patterns observed in plants carrying a class I state of a_2^{m-1} may suggest why this was so. Only when the behavior of the state of a_2^{m-1} belonging to the second class was being examined was initial evidence obtained of the presence in this system of a heritable element responsible for suppressing phenotypic expression of a_2^{m-1}. In this aspect of its operation, it resembled the *Spm* element previously discovered in the a_1^{m-1} cultures. We now know that this resemblance is more than coincidental. These elements are basically alike, and it is altogether probable that they arose from a common progenitor. Each appears to represent a different state of one controlling element. The following discussions will consider the differences between them, and will explain the confusion experienced in earlier attempts to interpret the operation of the system responsible for controlling gene expression at a_2^{m-1}.

After it was recognized that an *Spm*-type element was involved in this system, the *Spm* element extensively examined in the a_1^{m-1} cultures was introduced into some plants having one of the states of a_2^{m-1} belonging to class I, and subsequently into plants carrying other states of this class. With this *Spm* element present, the mode of control of gene expression was as sharply revealed as it had previously been in the case of a_1^{m-1}. Moreover, some states of a_2^{m-1} were seen to resemble some states of a_1^{m-1}. In the absence of *Spm,* both plants and kernels having one of these class I states of a_2^{m-1} were pigmented. The intensity of anthocyanin pigmentation in plants was rather high, whereas in kernels it was always low; and the distribution of pigment was uniform in both plant and kernel. In the presence of *Spm,* no pigment appeared except in those areas of plant or kernel that arose from cells in which mutation to or toward A_2 had occurred. Such mutations occurred in some germinal cells, and, as a consequence, mutants could be isolated. They were found to be stable in expression in subsequent generations of plants, either in the presence or in the absence of *Spm*.

Through the use of this *Spm* element, it was learned that class I states of a_2^{m-1} differ from one another in the manner in which they control the time and frequency of occurrence of mutation during development of plant or kernel; and some sharply expressed distinctions among them were noted. These class I states of a_2^{m-1} and the states of a_1^{m-1} are much alike. The similarities include the appearance of uniformly distributed anthocyanin pigment in plant and kernel, and stability of this phenotypic expression in successive generations of plants, in the absence of *Spm;* suppression of all visible evidence of this kind of gene action in the presence of *Spm;* and also control of mutation type and pattern by the state when *Spm* is present. The *Spm* element originally present in the a_2^{m-1} cultures, on the other hand, did not always effect suppression of gene action in all parts of a plant or kernel. Mutations, however, occurred only in those parts of plant or kernel in which gene expression was suppressed. The pattern of mutation produced by any one state was not set, and the types observed ranged from early-occurring mutations, which resulted in the appearance of large areas of mutant phenotype, to late-occurring mutations, which produced only specks of deep pigmentation. Invariably the mutations were confined to regions in which the background

phenotype was nonpigmented, that is, in which the suppressive component of *Spm* activity was evident.

It is now apparent that one of the major difficulties in analyzing the control system responsible for the many different patterns of a_2^{m-1} gene expression was occasioned by alternating phases of activity of the *Spm* element in the a_2^{m-1} cultures. But one other factor also contributed to the confusion. Early in the study of a_2^{m-1}, a new type of state appeared, whose expression was in great contrast to those observed with other isolates of a_2^{m-1}. It was therefore set apart and designated as the class II state of a_2^{m-1}. By means of various kinds of experiment with this state, it was first learned that the *Spm* element in the a_2^{m-1} cultures may undergo frequent changes in activity during the development of a plant, each such change affecting its capacity to serve as a suppressor-mutator. Clearly, some regulatory mechanism controls the time of occurrence of such changes, although it is not yet understood. Such a change may evidently occur within an individual cell during development, for well defined sectors in a plant or kernel often exhibit the result. The direction of change may be from active to inactive, or from inactive to active, and alternating cycles may also be observed. The class II state of a_2^{m-1} readily reveals these changes in action capacity of *Spm,* for, with this state, the *Spm* element in its active phase serves only to inhibit expression of gene action at a_2^{m-1}. No evidence of mutation has yet been obtained with this state, although abundant evidence has been obtained with the class I states. It is important to bear this fact in mind in order to appreciate the significance of the phenotypes arising from the class II state, which will be considered below. All the variegated patterns to be described reflect only changes undergone by the *Spm* element itself.

The class II state of a_2^{m-1}. When *Spm* is absent, or when an *Spm* element is present in its inactive phase, the class II state of a_2^{m-1} produces a phenotype similar to that given by standard A_2. Both kernel and plant are deeply pigmented. In a plant that is a_2^{m-1} (class II)/a_2 in constitution and carries a single active *Spm* element derived from the a_2^{m-1} cultures, suppression of the effects of a_2^{m-1} gene action is not complete. Pigment is present, but it is less intense than the pigment of plants having no *Spm* or *Spm* in its inactive phase. A change in *Spm* from an active to an inactive phase during the development of such a plant is made evident by a well defined area of very deep pigmentation in a more lightly pigmented background. The positions of such areas in a plant, their number, and their relative sizes reveal both time and frequency of occurrence of such changes in *Spm* activity during development. If the *Spm* element is inactive during early development, a change from the inactive to the active phase is represented by areas of lower pigment intensity in the background of intense pigmentation. In kernels, on the other hand, this *Spm* element in its active phase will suppress all expression of gene action at a_2^{m-1}. Those parts of the kernel in which it is active are totally colorless, but other parts, where it is inactive, are intensely pigmented. Changes in *Spm* action phase may alternate, and both the times and the types of change are revealed in the kernel phenotypes. In kernels having one *Spm* element, these alternating changes may be observed readily. For example, a large pigmented area may be seen in an otherwise colorless region of a kernel. Within this large pigmented area, smaller colorless areas may be observed, and within these, in turn, specks of deep pigmentation. In this illustration, the sequence of changes of phase of *Spm* activity during development of the kernel was from active to inactive to active, and again to inactive.

The dose of *Spm* can alter the patterns of variegation produced by the class II state, and this dose effect can be seen in

both plant and kernel. It is particularly well expressed in the aleurone layer of the endosperm of the kernel. The endosperm is triploid and therefore is useful for examining the effects produced by various doses of any chromosomal component. The female gametophyte contributes two genetically identical haploid nuclei to the primary endosperm nucleus, and the pollen grain contributes one haploid nucleus. The different patterns of variegation exhibited by kernels having one, two, or three active *Spm* elements provide a means of determining whether a plant has no *Spm* element or an *Spm* element in its inactive phase. Before explaining this method of differentiation, it is necessary to review some of the tests that have been made with the class II state of a_2^{m-1}.

The first example concerns a plant that carries this state and also carries one *Spm* element, which was in its active phase in the cells that produced a particular ear of the plant. The endosperm of any kernel appearing on that ear after pollination should have received from the female parent either no *Spm* or two active *Spm* elements; and the ratio of this distribution of *Spm* among the kernels is expected to be 1 to 1. If the plant serving as pollen parent for this ear is homozygous for a_2 and has no *Spm* element, half the a_2^{m-1}-carrying kernels produced by the cross should have no *Spm*, and half should have two *Spm* elements. Kernels of the former type should be deeply and uniformly pigmented, and those of the latter type should reveal the presence of the active *Spm* elements. In the many tests of this kind so far conducted, the *Spm*-carrying kernels have shown a number of rather small, deeply pigmented spots in a colorless background, and the ratio of fully pigmented kernels to variegated kernels has been 1 to 1. When the reciprocal cross is made, a 1-to-1 ratio of deeply pigmented to variegated kernels again results. In this case, however, the variegated kernels show a number of large, deeply pigmented areas, within which smaller, colorless areas often appear. It has been learned from other tests, one of which will be described below, that this is the characteristic pattern of variegation in kernels that start development with one active *Spm* element in the primary endosperm nucleus.

In a cross similar to that described above except that *Spm* is absent in the ear-producing parent, all the a_2^{m-1}-carrying kernels on the resulting ears should be deeply and uniformly pigmented, for none of them will have an *Spm* element. If, however, the pollen parent that is homozygous for a_2 has one active *Spm* element, half its pollen grains should have no *Spm* and half should have one *Spm*. Two types of a_2^{m-1}-carrying kernels are expected to appear on an ear produced by this cross, and they should be present in equal numbers. Those with no *Spm* should be deeply and uniformly pigmented, and those with one *Spm* should be variegated. More than fifty crosses of this kind were made, and all the ears so produced had a 1-to-1 ratio of uniformly pigmented to variegated kernels. In all cases, the variegated kernels exhibited pigmented spots in a colorless background; many of these spots were large. Within most of the large pigmented areas, smaller colorless spots were present, and some of these contained specks of pigment.

If pollen from a plant homozygous for a_2 and carrying one active *Spm* element is placed on the silks of an ear of a plant homozygous for the class II state of a_2^{m-1} and carrying one *Spm* element that was in its active phase in the cells that gave rise to the ear, then four types of kernels appear in equal proportions. One type is fully pigmented (no *Spm*); another shows many pigmented areas, many of them large, in a colorless background (one *Spm*); a third has pigmented spots, all rather small, in a colorless background (two *Spm*); and a fourth shows only small specks of pigment in a colorless background (three *Spm*). Higher doses of *Spm* elements in the endosperm have been produced by other kinds of testcrosses.

These tests have shown that kernels having four *Spm* elements exhibit at the most one or a few tiny specks of deep pigmentation; much more often they are totally colorless. Kernels having more than four *Spm* elements are all totally colorless.

The relation between pattern of variegation and dose of *Spm* was confirmed by tests in which the location of *Spm* in the chromosome complement was known because of its linkage with some genetic marker. The distribution of the linked marker among the various categories of variegated kernels was that expected in the event of a direct relation between pattern of variegation and dose of *Spm;* an illustration will be given later. Confirmation was also obtained in tests of the *Spm* constitution of plants derived from the variegated kernels of each category.

As was mentioned earlier, the relation between pattern of variegation and dose of *Spm* has made it possible to determine whether a plant whose phenotype exhibits no evidence of *Spm* actually has no *Spm* or carries an *Spm* element in an inactive phase. This procedure will now be described.

Among a_2^{m-1}-carrying plants, those having an inactive *Spm* element and those having no *Spm* element may be phenotypically the same. *Spm* may be present although no evidence of it appears in any part of the plant. In other plants, there is no sign of the presence of *Spm* in the main stalk but one or more of the tillers have sectors of the phenotype produced by an active *Spm* element. Several kinds of test may be employed to determine whether or not *Spm* is present in a plant, or parts of a plant, where it is not revealed phenotypically. The most significant of these was formulated after it was discovered that the introduction of an active *Spm* element into a nucleus carrying inactive *Spm* elements will result in activation of the latter. In this test, ears for crosses are selected in plants showing no evidence at all of *Spm,* or in parts of a plant that show no evidence of *Spm* although it is known to be present in other parts of the plant. When possible, the first and second ears produced by the main stalk are used.

The silks of one ear of a plant receive pollen from a plant that is homozygous for a_2, and for other recessive markers if these are useful for the test, but that carries no *Spm*. If *Spm* is absent in the female parent, or if it is present but was in its inactive phase in the cells that gave rise to the ear used in the cross, all the a_2^{m-1}-carrying kernels on this ear will be deeply and uniformly pigmented. Silks of a second ear of the same plant receive pollen from a plant of the same constitution as the first pollen parent except that it carries one active *Spm* element. Half its pollen grains will carry an active *Spm* element, and half will have none. If *Spm* is absent in the female parent, two types of a_2^{m-1}-carrying kernels will appear on the second test ear, in equal frequencies. One type will have deep pigmentation, uniformly distributed over the aleurone layer (no *Spm*). The other type will be variegated, with a colorless background and many pigmented areas in a pattern that characteristically appears when only one active *Spm* is present. If the ear-producing parent carries an inactive *Spm* element, however, three instead of two types of kernel will appear on the ear produced by this second cross. Half the kernels will be deeply and uniformly pigmented; the other half will be variegated, and will fall into two sharply defined subclasses with equal numbers of kernels in each. One subclass will show the heavily variegated pattern characteristically produced when one active *Spm* element is present, and the other will show the pattern produced when three active *Spm* elements are present. All the variegated kernels receive one active *Spm* element from the pollen parent. The differences in pattern among them discriminate between those that receive no *Spm* from the female parent (the one-*Spm* pattern) and those that receive two inactive

Spm elements from the female parent (the three-*Spm* pattern). The three-*Spm* pattern appears because the two inactive *Spm* elements derived from the female parent are activated by the *Spm* derived from the male parent. Among the fully pigmented kernels, half receive no *Spm* from the female parent and half receive the two inactive *Spm* elements. In the latter category, the *Spm* elements remain inactive during the development of the kernel, for no active *Spm* element is introduced into the primary endosperm nucleus by the male parent to trigger them into activity.

Evidence to confirm the above analyses of *Spm* constitution among kernels of different types was derived from tests of a_2^{m-1}-carrying plants in which one inactive *Spm* element was present and was located near *wx* in one of the two chromosomes 9; the homologous chromosome 9 carried *Wx*. The results of tests conducted with one plant may be examined for illustrative purposes. Two ears of a plant that was a_2^{m-1} (class II) *Bt*/a_2 *bt*, *Wx* +/*wx Spm* (inactive) were crossed by a plant homozygous for a_2, *bt*, and *wx* and carrying no *Spm* element. (The locus of *Bt*, normal endosperm, and its recessive allele, *bt*, brittle endosperm, is close to the centromere in the long arm of chromosome 5, and it undergoes approximately 6 per cent crossing over with the locus of a_2^{m-1} in the short arm of that chromosome.) None of the a_2^{m-1}-carrying kernels on these two ears was variegated. All the kernels were deeply and uniformly pigmented, and the ratio of *Wx* to *wx* among them was 1 to 1.

A third ear of this plant was crossed by a plant that, again, was homozygous for a_2, *bt*, and *wx*, but had one active *Spm* element. (Twenty-eight testcrosses had been made to determine the presence and activity of the *Spm* element in this pollen parent. Among 5997 kernels produced in these tests, 3041 had received no *Spm* element from the pollen parent and 2956 had received an active *Spm* element. It could therefore be assumed that half the pollen grains of this plant had no *Spm* and half carried an active *Spm* element.) When pollen of this plant was used on the third ear of the plant under consideration, 468 kernels were produced. Among them, 245 were completely colorless—34 *Bt* (18 *Wx* : 16 *wx*) and 211 *bt* (105 *Wx* : 106 *wx*). Undoubtedly, these were homozygous for the stable recessive, a_2. The remaining 223 kernels (195 *Bt* : 28 *bt*) had pigment and so had received a_2^{m-1} from the female parent. Among them, 114 were deeply and uniformly pigmented (57 *Wx* : 57 *wx*); the other 109 were all variegated, with pigmented areas in a colorless background. In 57 of these, the pattern of variegation was characteristic of the presence of one *Spm;* 45 of them were *Wx* and 12 were *wx*. The other 52 variegated kernels had only specks of pigment in a colorless background, the characteristic pattern produced when three *Spm* elements are present; only 9 of these were *Wx*, whereas 43 were *wx*.

The ratio of kernel types on this third ear, and the relation between pattern type and the alleles of *Wx* among the variegated kernels, indicated that the female parent had some component in the *wx*-carrying chromosome, and closely linked with *wx*, that was responsible for change of pattern from a one-*Spm* type to a three-*Spm* type. That this was the *Spm* element in its inactive phase was indicated by two kinds of test. One was conducted with sib plants and with related plants in which an *Spm* element was obviously present and active. These plants were either a_2^{m-1} *Bt*/a_2^{m-1} *Bt* or a_2^{m-1} *Bt*/a_2 *bt*, and all were *Wx*/*wx*. The silks of ears of these plants received pollen from plants homozygous for a_2, *bt*, and *wx* but having no *Spm;* and this set of testcrosses produced a total of 3007 a_2^{m-1}-carrying kernels. In 1538 of them, the aleurone layer was deeply and uniformly pigmented; 1345 of these were *Wx* and 193 were *wx*. The remaining 1469 kernels were variegated, with pigmented areas in a colorless background; 166 were *Wx* and 1303 were *wx*. Relatively close linkage of *Spm*

with *wx* was demonstrated by the ratios of kernels on each of the ears.

The second kind of test confirming the presence of an *Spm* element in its inactive phase was much more direct. It was made with sib plants of the one considered above. In them, the phenotype of the main stalk showed no signs of *Spm*, but one of the tillers gave obvious evidence of its presence, in an active phase. The two kinds of cross described above were conducted with ears of the main stalk on such plants. The results in each case were similar to those described: no evidence of *Spm* when the pollen parent contributed no *Spm*, and evidence of an inactive *Spm*, closely linked to *wx*, when the pollen parent contributed *Spm*. The presence in the main stalk of an inactive *Spm* element, closely linked with *wx*, was inferred. Confirmation came from tests of the constitution of tiller ears of such plants. They were used in crosses with a plant homozygous for a_2, *bt*, and *wx*, in which no *Spm* was present. Segregation of kernel types on these ears clearly indicated that the *Spm* element in the tiller that produced each of them was located close to *wx* in one chromosome 9. There appears to be little doubt, then, of the effectiveness of the described tests in revealing whether *Spm* is absent in a plant or is present in its inactive phase. The tests have also shown that a change in phase of *Spm* is not associated with a detectable change in its location in the chromosome complement. Evidence of transposition of the *Spm* element has been obtained; but the observed changes in location have not altered the expression of its cyclically occurring changes in action phase.

Tests of Spm activity in plants having class I states of a_2^{m-1}. The deeply pigmented areas in a colorless background observed in kernels having the class II state of a_2^{m-1} and an active *Spm* element are an expression of events that affect only the *Spm* element. They do not express mutations to stable A_2, as do the deeply pigmented areas that appear in kernels having a class I state of a_2^{m-1}. Therefore, the class II state has been particularly useful in elucidating changes in phase of activity of the *Spm* element, as described in the previous section. Nevertheless, similar analyses may be conducted with the class I states of a_2^{m-1} if attention is focused on the patterns of palely pigmented areas that may also be present in kernels exhibiting the deeply pigmented spots that represent mutation-producing events at a_2^{m-1}.

As was stated earlier, kernels carrying class I states of a_2^{m-1} have uniformly pale pigmentation if *Spm* is absent. The same phenotype will appear if *Spm* is present but in an inactive phase. Thus, the pale-pigmented phenotype in the kernels carrying the class I states of a_2^{m-1} is the counterpart of the deeply pigmented phenotype in kernels having the class II state. The relation of pattern of these pale-pigmented areas to dose of active *Spm* elements is the same as that of the deeply pigmented areas. By observing these patterns in kernels having a class I state, therefore, it has been possible to conduct the same kinds of test as those described above, to distinguish between absence of *Spm* and its presence in an inactive phase.

In kernels having a class I state, then, two distinctly different types of variegated pattern appear: deeply pigmented areas, arising from mutation, which are solidly pigmented throughout; and pale areas, which reflect inactivations of *Spm*. When a pale area is large (one-*Spm* pattern) it usually contains smaller colorless areas within it, and some of these, in turn, may exhibit small specks of either the mutant phenotype or the pale phenotype. Mutant spots, it has been noted, always appear in a part of the kernel where *Spm* has been active in ancestor cells; they do not appear in the pale areas. In other words, mutations at a_2^{m-1} occur only in those cells in which *Spm* is in its active phase.

Examination of kernels carrying the class I states has also shown that the size of mutant spots is always small if activity of the *Spm* element commences late in

development. Thus, if a change in *Spm* from an inactive to an active phase is delayed until late in the development of a tissue, only small spots of mutant phenotype will appear in the areas in which *Spm* is active, even when the state that is present is known to be one that would produce many large areas of mutant phenotype, as a result of early-occurring mutations, if the *Spm* element were in its active phase during early development. Therefore, the size of a mutant spot produced by class I states of a_2^{m-1} is conditioned not only by the particular state itself but also by the timing of change in phase of activity of the *Spm* element.

Knowledge of the activity cycles of the *Spm* element in the a_2^{m-1} cultures has clarified many of the bewildering aspects of a_2^{m-1} behavior encountered early in its study. Its seemingly disorderly patterns of gene expression and apparently unorthodox types of inheritance behavior can now be interpreted and no longer give cause for confusion. The conditions responsible for change in activity of *Spm* are not yet understood, but studies are under way that may help elucidate them.

Before leaving this discussion, it may be useful to point out the resemblance between the patterns of pigmented spots, arising from inactivations of *Spm*, that appear with different doses of *Spm* and those that are produced by different doses of *Ac*. In the former case, the pattern is produced by change in phase of activity of *Spm*, whereas in the latter case it is produced by mutation-inducing events instigated by *Ds*, the complementary component of the *Ds-Ac* system. The resemblance of the two systems with respect to pattern type and dose effect is so great that it suggests some basic similarity in mode of operation.

A Modifier Element in the a_1^{m-1}-Spm System

When the standard *Spm* element of the a_1^{m-1} cultures is present, mutations occur at the a_1^{m-1} locus in plants and kernels; the size and number of mutant areas reflect both the time of occurrence of mutation during development and the number of cells in which it takes place. As described earlier, the different states of a_1^{m-1} are recognized by the distinctive patterns of mutation they produce; and the expression of a state of a_1^{m-1} is not altered by dose of *Spm*, for the same pattern appears when one or more standard *Spm* elements are present.

During the course of study of a particular state of a_1^{m-1}, a single kernel appearing on one ear of a plant reflected a mutation frequency much higher than that expected of the state that was present in the plant. The plant grown from this kernel likewise exhibited a marked increase in mutation frequency. From testcrosses of this plant and examination of the progeny it became evident that a modifier element, independently located in the chromosome complement, was responsible for the increase in mutation frequency, and that the action of the modifier was expressed only in kernels and plants that also carried *Spm*. Further examination revealed that this modifier element underwent transposition. Preliminary findings about the modifier element were given in Year Book 56; and the investigation was continued this year, as reported below.

Effects of the modifier element on the expression of four different states of a_1^{m-1} were examined. When *Spm* is present, without the modifier, one of these states gives rise to a few, relatively late-occurring mutations; a second state produces more mutations, most of which occur relatively late in development; a third gives very many mutations, all occurring late in development; and the fourth produces many mutations, some of which occur early in development. It was found that the modifier alters the mutation patterns of only the first two of these four states; it does not change the patterns associated with the other two. In the two affected states, an

increase in frequency of mutations when the modifier is present is expressed in a stepwise manner.

The first-mentioned state, which gives relatively few mutations in the absence of the modifier, gives many more in its presence. The mutation pattern so produced resembles that of the second state when the modifier is absent. In turn, the effect of the modifier on the second state is to increase the mutation frequency so that the pattern of mutations resembles that of the third state described above.

In the testcrosses made with the first plant in which the modifier appeared, no linkage of the modifier with genetic markers in chromosomes 3, 5, or 9 was detected. In the progeny of this plant, however, it was early discovered that parts of some plants lacked the modifier and that other plants, or parts of plants, carried different numbers of modifier elements. Tests were conducted with a number of these plants in order to detect changes in location of the modifier that might place it in one of the three above-named chromosomes. It was detected in chromosome 3 in one plant, and in chromosome 9 in another. Tests were then carried out with some of the progeny of each of these two plants, to determine the constancy of location of the modifier.

One of the testcrosses made with a plant whose constitution was a_1^{m-1} Sh_2/a_1 sh_2 suggested that a modifier element was located in its a_1 sh_2-carrying chromosome 3. The state of a_1^{m-1} in this plant was the second of the four described above. In another test, pollen of this plant was placed on the silks of a plant homozygous for the second state of a_1^{m-1} and for Sh_2 but carrying no *Spm* or modifier element. This cross produced 356 kernels, classified as follows: 1 deeply and uniformly pigmented (germinal mutation); 206 uniformly pale colored (no *Spm*); 65 variegated, with a number of small, deeply pigmented spots in a colorless background (*Spm*, no modifier); and 84 variegated, with very many more mutant spots (*Spm* and modifier).

The silks of ears produced by 11 plants derived from kernels in the last category received pollen from plants homozygous for the second-described state of a_1^{m-1} and for sh_2 but having no *Spm* or modifier element. Eight of these 11 plants were a_1^{m-1} Sh_2/a_1 sh_2 in constitution, and the other 3 were homozygous for a_1^{m-1} and Sh_2. The modifier element was present in all 8 plants having the former constitution; some plants had one modifier element and others had two, but each carried a modifier element in the a_1 sh_2 chromosome 3. In the cells that gave rise to the tested ears of 5 of these 8 plants, a single modifier element was present. A single *Spm* element was also present in these 5 plants, and was inherited independently of the modifier element (table 9, A). The

TABLE 9. *Spm* and Modifier Constitution of Different Plants of a Culture, as Determined by the Cross a_1^{m-1} Sh_2/a_1 sh_2, *Spm*, modifier ♀ × a_1^{m-1} sh_2/a_1^{m-1} sh_2, no *Spm*, no modifier ♂

Modifier Elements, location and number	*Spm* Elements, number	Plant No.	No. of Ears Tested per Plant
Group A			
a_1^{m-1} Sh_2 +/a_1 sh_2 *Mod*	1	2	1
		4	3
		5	1
		7	1
		8	1
Group B			
a_1^{m-1} Sh_2 +/a_1 sh_2 *Mod*, plus independently located modifier	1	1	1
		3	1
Group C			
Same as B	2	6	2

cells that produced the tested ears of 3 other plants had two modifier elements; one was located in the a_1 sh_2-carrying chromosome, and the location of the other was not determined. The cells that gave rise to the tested ears of two of these plants contained a single *Spm* element (table 9, B), and those that produced the two tested ears of the third plant had two independently located *Spm* elements (table 9, C). The frequencies of the different types of kernels on the testcross ears, which revealed these

three types of constitution, are given in table 10. In A of table 10, the percentage of recombination between sh_2 and the modifier is 17.2. This value need not represent the percentage of crossing over between sh_2 and the modifier, for some of the kernels in the recombinant classes probably reflect the consequence of independent meiotic distribution of the modifier and sh_2 in some sporocytes in which the modifier had been transposed to a new location in an ancestor cell.

The modifier element located in chromosome 9 was detected when an ear of a plant of the constitution a_1^{m-1} Sh_2/a_1 sh_2, in either chromosome 3 or chromosome 9. A modifier was present, located in the *Wx*-carrying chromosome, in at least some part of 6 of these plants. In the 7th (plant 5, table 11), the results of the testcross, made with the ear of the main stalk and two tiller ears, indicated that the cells producing each of these three ears had one modifier element, but that it was not located in chromosome 9, or, at any rate, not close enough to *Wx* to show evidence of linkage with it.

A similar situation was found with respect to the ear of the main stalk in plant 1, table 11, although tests of the tiller ear of

TABLE 10. Types of Kernels Appearing on Ears Produced by Plants Whose Constitutions Are Entered in A, B, and C of Table 9

Constitution	Phenotype of Kernel								Totals
	Deeply Pigmented (Germinal Mutation)		Uniformly Pale Colored (No *Spm*)		Spots of Deep Pigmentation in Colorless Background (*Spm* Present)				
					Few Spots (No *Mod*)		Many Spots (*Mod*)		
	Sh_2	sh_2	Sh_2	sh_2	Sh_2	sh_2	Sh_2	sh_2	
A	15	0	531	548	479	107	92	476	2248
B	5	0	193	166	67	21	89	141	682
C	4	0	78	89	95	21	112	192	591

Wx/wx was used in a cross with a plant homozygous for a_1^{m-1}, Sh_2, and *wx* and having no *Spm* or modifier element. The cells that gave rise to this ear had one *Spm* and also one modifier, the latter located in the *Wx*-carrying chromosome. Kernels from this ear that were *Wx* and showed the presence of both *Spm* and the modifier were used to grow 8 plants, which were tested for *Spm* number and for modifier number and location by means of crosses with plants homozygous for a_1^{m-1}, sh_2, and *wx* and having no *Spm* or modifier element. One of the 8 plants proved to have no *Spm* element, and therefore the presence of the modifier could not be detected by this cross. In all examined parts of the other 7 plants, however, one *Spm* element was present, and it was not linked with markers the plant and also of the pollen of this tiller indicated that the modifier was linked with *Wx* in at least that part of the plant. In a tiller ear of plant 6, no modifier element was present; and absence of the modifier was also observed in part of a tiller ear of plant 2. In plant 2, the cells that gave rise to the main ear carried two modifier elements, one of which was linked with *Wx*. The distribution of kernel types derived from the testcrosses of plants 1 to 7 is given in table 12. Linkage of the modifier element with *Wx* is clearly expressed by the data of parts A and B of the table; but absence of such linkage is evident from the results of the tests of the three ears produced by plant 5 (part C). The types of kernels appearing on the tiller ear of plant 2 are recorded in part E.

TABLE 11. Number and Location of Modifier Elements in the Progeny of a Plant Having One Modifier Element Linked with *Wx* in Chromosome 9

Plant No.	Modifier Elements in Different Parts of Individual Plants			
	A *Wx Mod/wx* +	B *Wx Mod/wx* +; 1 *Mod* Independently Located	C *Wx/wx*; 1 *Mod* Not Linked with *Wx*	D *Wx/wx*; No *Mod*
1	Ear of tiller Pollen of tiller		Main ear	
2	Tiller ear *	Main ear		
3	Main ear Tiller ear			
4	Main ear			
5			Main ear Ears of 2 tillers	
6	Main ear Pollen of main stalk			Tiller ear
7	Main ear Tiller ear			

* A sector of this ear had no modifier element. See part E, table 12.

TABLE 12. Types of Kernels Appearing on Ears Produced by Plants Whose Constitutions Are Entered in Columns A to D, Table 11

Constitution Shown in Table 11, Column	Parentage in Cross	Phenotype of Kernels								Totals
		Deeply Pigmented (Germinal Mutation)		Pale Colored (No *Spm*)		Spots of Deep Color in Colorless Background (*Spm* Present)				
						Few Spots (No *Mod*)		Many Spots (*Mod*)		
		Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*	*Wx*	*wx*	
A	♀	14	17	498	490	150	388	364	109	2030
	♂	18	17	354	330	125	241	238	69	1392
B	♀	6	5	77	89	6	31	90	48	352
C	♀	2	5	215	229	99	119	98	102	869
D	♀	0	2	77	55	56	62	0	0	252
E *	♀	3	0	48	63	15	34	35	23	221
E †		0	1	17	19	29	22	0	0	88

* Tiller ear of plant 2; modifier present.
† Sector with modifier absent.

In one sector of this ear the modifier was absent. The cells producing the remainder of the ear carried a single modifier element, which appears to have been in the *Wx*-carrying chromosome, although the data are few and the expression of linkage is less marked than in the results entered in part A of the table.

The evidence outlined above, together with that obtained in other studies of the modifier, indicates that this element, like the elements *Spm, Ds, Ac,* and so forth, may undergo transposition from one location to another in the chromosome complement, at a time either early or late in the development of a plant. Another finding of the past year is that the expression of the modifier is the same whether the standard *Spm* or *Spm-w* is present. (For differences in effect produced by these two states of *Spm,* see Year Book 56.) In the absence of the modifier, on the other hand, the distinct pattern of mutation at a_1^{m-1} effected by each of these two states of *Spm* is clearly expressed. The difference may be observed among the kernels on ears of plants carrying both states of *Spm* as well as the modifier, when such ears are used in the testcross described above. Those kernels having one or the other state of *Spm* but no modifier are readily distinguished from each other, whereas kernels with either state of *Spm* and also the modifier cannot be distinguished from one another. Thus, the modifier element appears to be a component of the *Spm* system that can modify the expression both of a state of a_1^{m-1} and of the *Spm* element.

Continued Investigation of Transposition of Spm

A report was given in Year Book 56 of two progeny tests made to determine the time and frequency of transposition of the standard *Spm* element carried in the a_1^{m-1} cultures. The ear-producing parent in one of these tests had one *Spm,* located in one of its chromosomes 9. The numbers of *Spm* elements present in different plants of the progeny, and in different parts of a single plant, and also the location of *Spm* with reference to the marker *Wx,* carried in chromosome 9, were summarized in table 6, page 394, Year Book 56. The tests reported there were extended this year, with plants derived from variegated, *Wx* kernels on five of the ears recorded in that table: the second and tiller ears of plant 7285A-1, a tiller ear of plant A-2, a tiller ear of plant A-7, and the main ear of plant B-6. The cells that gave rise to four of these ears had one *Spm* element, located in the *Wx*-carrying chromosome 9. The cells that gave rise to the fifth ear (tiller ear of plant A-1) carried one *Spm;* but it was not linked with *Wx,* even though testcrosses of both the first and second ears of the main stalk of this plant showed linkage of *Spm* with *Wx.* The plants studied last year provided a number of examples of transposition of *Spm* occurring early in development. The tests conducted this year were made for two purposes: first, to learn whether or not early-occurring transposition of *Spm* would continue to be expressed in the progeny plants; and second, to learn whether or not the transposition of *Spm* away from a known location in chromosome 9 might be followed later by transposition back to its former location.

To carry out the first of these purposes, *Spm* constitution and location were determined in 37 progeny plants. Each plant was grown from a variegated, *Wx* kernel on an ear produced from cells in which *Spm* had been located in the *Wx*-carrying chromosome 9. Twelve plants were derived from one ear, 8 plants each from two other ears, and 9 plants from a fourth ear. Table 13 summarizes the results of these tests, which have been incorporated into a single table because early-occurring transposition of *Spm* was observed among the plants of all four cultures. It may be seen from the table that early-occurring transposition of *Spm,* evident in the parent

plants, was likewise exhibited in their progeny.

To determine whether or not sequential transpositions of *Spm* may be directed in such a way that removal of *Spm* from a known location in chromosome 9 may be followed by a return to that location, tests of *Spm* number and location in different plants and different parts of the same plant were conducted with 11 plants derived from the variegated, *Wx* category of kernels on the tiller ear of plant 7285A-1, mentioned above. In this tiller, the *Spm* element had been transposed away from a location close to *Wx* in chromosome 9. Twenty-one ears in all were obtained from these 11 plants. Among them, there were six certain cases of successive transpositions of *Spm;* but in none of the ears was *Spm* found to be linked with *Wx*. Although this test is limited, it does indicate that a directed type of transposition of *Spm,* which returns it to the location it previously occupied, does not occur regularly.

TABLE 13. *Spm* Constitution and Location in Different Plants and in Different Parts of Individual Plants

No. of Ears Tested per Plant	Position of Ear in Plant	No. of Plants	*Spm* Constitution and Linkage with *Wx*	No. of Plants with Given Constitution
1	1st ear, main stalk	13	1 *Spm,* linked with *Wx*	9 *
			2 *Spm,* neither linked with *Wx*	3
			No *Spm*	1
2	1st and 2nd ear, main stalk	1	1 *Spm,* linked with *Wx*	1
2	1st ear, main stalk Tiller ear	15	1 *Spm,* linked with *Wx,* in both ears	10
			1 *Spm,* linked with *Wx,* in 1st ear; 1 *Spm,* not linked with *Wx,* in tiller	1
			1 *Spm,* linked with *Wx,* in 1st ear; 2 *Spm,* one linked with *Wx,* in tiller	1
			2 *Spm,* one linked with *Wx,* in both ears	1
			1 *Spm,* not linked with *Wx,* in both ears	2
3	1st and 2nd ear, main stalk Tiller ear	6	1 *Spm,* linked with *Wx,* in all three ears	3
			1 *Spm,* linked with *Wx,* in 1st and 2nd ears, main stalk; 2 *Spm,* neither linked with *Wx,* in tiller	1
			2 *Spm,* one linked with *Wx,* in 1st ear; 1 *Spm,* linked with *Wx,* in 2nd ear; no *Spm* in tiller	1
			2 *Spm,* one linked with *Wx,* the other linked with *Pr,* in all three ears	1
3	1st ear, main stalk Ear on each of two tillers	2	1 *Spm,* linked with *Wx,* in all three ears	2

* One ear with sector in which *Spm* was absent.

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